

RNA-Based Drug Delivery, Current Advances, Pharmaceutical Applications, and Future Perspectives

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Abstract

RNA-based therapeutics have emerged as a transformative approach in modern medicine due to their ability to regulate gene expression with high specificity and precision. Advances in molecular biology, RNA chemistry, and nanotechnology have accelerated the development of diverse RNA modalities, including messenger RNA (mRNA), small interfering RNA (siRNA), microRNA (miRNA), antisense oligonucleotides (ASOs), and RNA aptamers. These therapeutics offer promising strategies for the treatment of cancer, genetic disorders, infectious diseases, neurological conditions, and cardiovascular diseases. However, the clinical translation of RNA therapies is challenged by issues such as poor stability, rapid degradation by nucleases, limited cellular uptake, immunogenicity, and off-target effects. Recent innovations in delivery technologies, particularly lipid nanoparticles, viral vectors, extracellular vesicles, polymeric carriers, and other nanomaterial-based systems, have significantly improved the safety and efficacy of RNA delivery. The successful application of mRNA vaccines during the COVID-19 pandemic has further highlighted the therapeutic potential of RNA platforms. This review summarizes the different classes of RNA therapeutics, their mechanisms of action, pharmaceutical applications, and the latest advances in delivery systems. Additionally, current challenges and future perspectives in the field are discussed, emphasizing the need for targeted, tissue-specific, and personalized RNA-based treatment strategies. Continued progress in RNA engineering and delivery technologies is expected to expand the clinical utility of RNA therapeutics and establish them as a cornerstone of precision medicine.

Keywords: RNA therapeutics, RNA-based drug delivery, mRNA, siRNA, antisense oligonucleotides, lipid nanoparticles, nanocarriers, gene therapy, precision medicine, pharmaceutical applications

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INTRODUCTION

Recent advances in the synthesis, modification, stability, and cellular transport of ribonucleic acid (RNA) have shown promise for the development and advancement of RNA-based treatments [1]. Novel therapeutic approaches that target diseases at the genetic level have been developed recently because of advances in molecular biology and pharmaceutical sciences; among these approaches, RNA-based therapeutics have emerged as a promising class of medicines capable of regulating gene expression and cellular functions [2, 3]. The biological understanding of RNA has changed from being merely an intermediary between DNA and protein to a dynamic and adaptable molecule that controls the functions of genes and cells in all living things since the groundbreaking discoveries of catalytic RNAs in the early 1980s and RNA interference in the late 1990s [4, 5]. The antisense

oligonucleotides (ASOs) inhibiting protein synthesis in the early 1980s promoted the rapid advances of RNA-based therapeutics [6]. In the 2000s, the proposal of RNAi and the use of siRNA silencing the human gene led to increased funding for RNA therapeutics [7]. Unlike conventional drugs that mainly interact with proteins or receptors, RNA-based therapies act by modifying RNA translation or interfering with specific genetic sequences, thereby offering a more precise mode of treatment [8, 9]. RNA-based therapeutics encompass a range of methods for treating diverse medical diseases. With uses in vaccines, cancer immunotherapy, and genetic disorders, messenger RNA (mRNA) treatment transports synthetic mRNA to generate proteins and trigger immune responses [10]. While antisense oligonucleotides (ASOs) and microRNAs (miRNAs) alter gene expression and may be used to treat illnesses such as viral infections, cancer, and genetic abnormalities, small interfering RNA (siRNA) silences particular genes [11, 12]. Numerous RNA-based treatments that expand the range of “drugable” targets beyond the reach of current pharmaceutical medications have emerged because of these and related discoveries [13]. Additional RNA molecule regulators and associated processes have also been thoroughly described. Numerous RNA-based drugs have been approved thus far, and many more are undergoing phase III trials (Figure 1) [14]. Because of their unique physicochemical and physiological characteristics, RNA-based treatments are more promising than traditional protein-targeted and DNA-based medications [15].

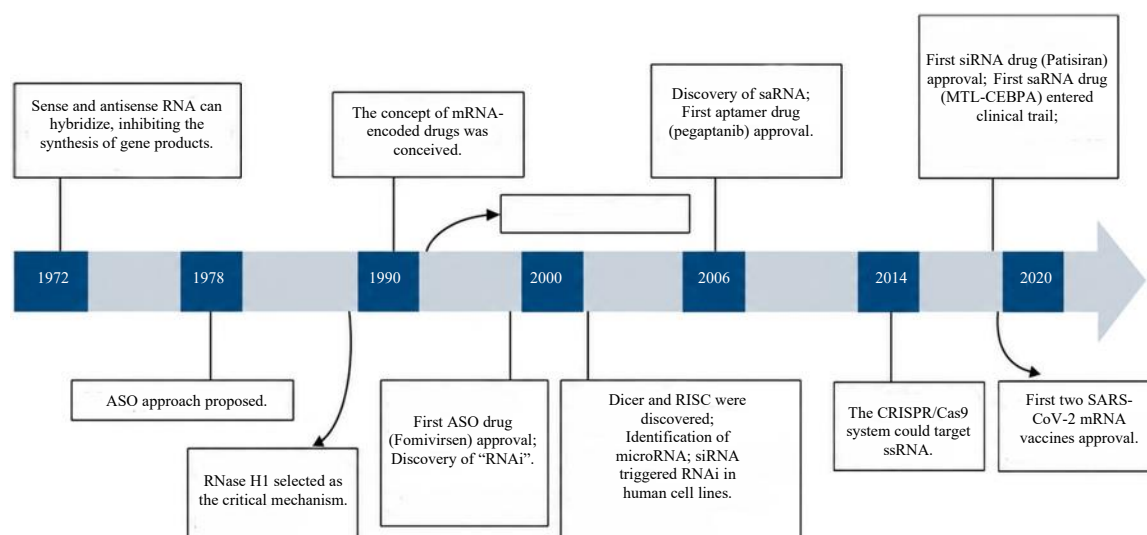


Figure 1. An overview of major developments in the RNA-targeting field. A timeline of RNA-based technological advances, drug approvals and other important events are highlighted. Rapid development in the RNA-targeting field has been applied to treat rare and common diseases. ASOs antisense oligonucleotides, RNAi RNA interference, RISC RNA-induced silencing complex, siRNA small interfering RNA, saRNA small activating RNAs, ssRNA single-stranded RNA.

Many types of therapeutic RNA, such as microRNA, antisense oligonucleotides, small interfering RNA (siRNA), and messenger RNA (mRNA), have been thoroughly studied for their possible uses in a variety of illnesses [16–18]. Inhibitors of mRNA translation (antisense), agents of RNA interference (RNAi), catalytically active RNA molecules (ribozymes), and RNAs that bind proteins and other molecular ligands (aptamers) are examples of RNA-based therapies that can be categorized based on their mode of action.

Over 50 RNA or RNA-derived therapies have made it to clinical development despite several obstacles along the road. Improvements in synthetic and natural nucleic acid carriers as well as the creation of chemically modified oligonucleotides have been prompted by issues with the distribution, specificity, stability, and immune activation of RNA therapies [19]. Phosphorothioate (PS) backbone modification; 2'-O-methyl (2'-OMe), 2'-fluoro (2'-F), and 2'-O-methoxyethyl (2'-MOE) sugar

substitutions; 2'-O, 4'-C-methylene linked bicyclic ribonucleotides known as a locked nucleic acid (LNA); and L-RNA (enantiomer of natural RNA) ONs known as spiegelmers (Figure 2) [20].

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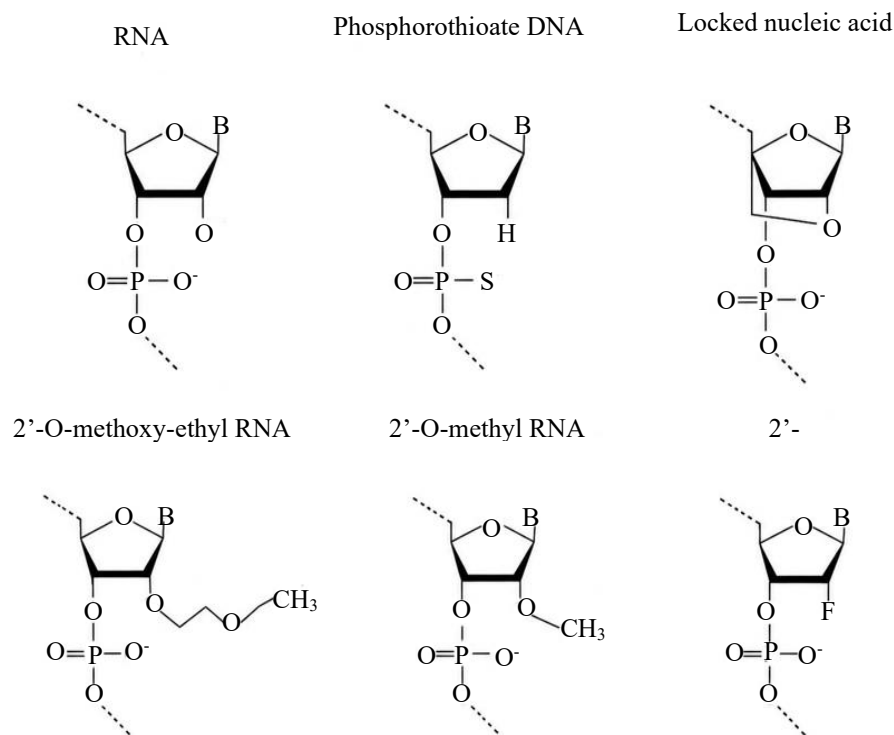


Figure 2. Common chemical modifications of therapeutic nucleic acid analogs. The unmodified RNA structure is shown next to backbone (5'-phosphorothioate), LNA, and 2'-substitutions (2'-O-methoxy-ethyl, 2'-O-methyl, and 2'-fluoro).

These substances can either repress the expression of genes that cause disease or increase the synthesis of therapeutic proteins. RNA-based treatments have demonstrated promising outcomes in the treatment of metabolic disorders, cancer, infectious diseases, and genetic abnormalities [21–23]. RNA molecules are now safer, more effective, and more bioavailable thanks to these developments. However, issues with immunological reactions, off-target effects, and effective tissue-specific delivery still prevent them from being widely used [24–26].

TYPES OF RNA-BASED THERAPEUTICS

Small Interfering RNA (siRNA)

- Short, double-stranded RNA molecules that are intended to target particular mRNA sequences.
- Silence genes by binding to complementary mRNA and causing its breakdown.
- Stops the production of proteins that cause illness.
- Extensively investigated in genetic diseases, viral infections, and cancer treatment [27, 28].

MicroRNA (miRNA)

- Endogenous single-stranded RNA molecules that regulate genes after transcription.
- The goal of therapeutic treatments is to either block overexpressed miRNAs or replace defective miRNAs.
- Instead of total breakdown, control mRNA translation to suppress the formation of proteins.
- Demonstrate promise in neurological, cardiovascular, and cancer disorders [29, 30].

Antisense Oligonucleotides (ASOs)

- Target mRNA-complementary short synthetic single-stranded nucleic acid sequences.
- Bind to mRNA and either prevent it from functioning or encourage its breakdown.
- Reduce aberrant protein expression while maintaining a high degree of sequence specificity.
- Used to treat inflammatory, rare hereditary, and genetic illnesses [31, 32].

Messenger RNA (mRNA) Therapeutics

- Entail introducing synthetic mRNA into cells to generate medicinal proteins.
- Make use of the cell's built-in translation system.
- Avoid integrating into the host DNA, guaranteeing a safe and reversible method.
- Used in protein replacement treatment, cancer immunotherapy, and vaccine development [33, 34].

RNA Aptamers

- RNA sequences that are short and single-stranded and fold into distinctive three-dimensional structures.
- Bind with great affinity to target proteins or receptors.
- With less immunogenicity, they function similarly to antibodies.
- Utilized in drug delivery systems, targeted therapy, and diagnostics [35, 36].

MECHANISM OF ACTION OF RNA-BASED THERAPEUTICS

RNA-based treatments function by controlling RNA-level gene expression. These treatments mostly work by regulating the synthesis of proteins implicated in illness processes. Specific interactions between therapeutic RNA molecules and their target messenger RNA (mRNA) provide the basis of the overall mechanism.

General Mechanisms

- Appropriate delivery systems are used to deliver RNA-based treatments into target cells.
- The therapeutic RNA identifies and attaches to a particular mRNA sequence once it is inside the cell. The normal function of mRNA is disrupted by this binding.
- Consequently, either a necessary therapeutic protein is created, or the production of disease-causing proteins is decreased.
- Abnormal cellular activity is suppressed or corrected because of the overall effect [37, 38].

Type of Mechanism

siRNA, or small interfering RNA.

- Inside the cell, siRNA attaches itself to complimentary mRNA.
- Protein synthesis is stopped by the degradation of the attached mRNA.

MiRNA, or microRNA

- Target mRNA is interacted with by miRNA, which inhibits translation.
- As a result, there is less protein expression without total mRNA destruction.

ASOs, or Antisense Oligonucleotides

- Target mRNA sequences are directly bound by ASOs.
- This binding either prevents mRNA from functioning or encourages its degradation.

Therapeutics Using Messenger RNA (mRNA)

- Once within the cell, synthetic mRNA exploits the machinery of the cell to make therapeutic proteins. Without changing the genes, the generated protein aids in the therapy of illness [39–42].

DELIVERY FORMULATION WITH NANOCARRIERS

Since RNAs have significant negative charges and chemical modifications, it is necessary to develop an effective carrier to shield the carried RNA from the hazardous physiological environment. Advances

in materials science and nanotechnology offer benefits and potential solutions to the problems of oligonucleotide drug delivery, particularly for the requirements of intracellular delivery across biological barriers and membranes. Custom optimization of nanoparticle biophysical (e.g., size, shape, and chemical/material composition) and biological properties (e.g., targeted ligand functionalization), which enables a high degree of customisation of the delivery platform, is one of the main advantages of nanoparticle drug delivery systems. Here, we presented three newly developed innovative nanocarriers along with two popular delivery methods: lipid nanoparticles and cationic polymer-based polyplexes.

Lipid Nanoparticles (LNPs)

The most used delivery system for oligonucleotide medications is LNPs [43, 44]. Ionizable cationic lipids, cholesterol, phospholipids, and PEG-lipids make up LNPs (Figure 3B①). The main constituents are ionizable cationic lipids. By electrostatically attaching to negatively charged nucleic acids, cationic lipids create “lipoplexes,” which are frequently employed in vitro for nucleic acid transfections (e.g., Lipofectamine™ RNAiMAX transfection reagents). Cholesterol, phospholipids, and helper lipids increase formulation stability and distribution effectiveness [45]. PEG-lipid can regulate particle size, inhibit particle aggregation, and extend the duration of in vivo circulation [46]. The sophisticated formulation of LNPs’ optimizing properties and production techniques has been well covered in other reviews [47]. LNP-mediated siRNA delivery is one example [48].

Cationic Polymer-Based Polyplexes

Standard formulations for the delivery of nucleic acids are called polyplexes. Cationic polymers and negatively charged nucleic acids combine electrostatically to create them spontaneously (Figure 3B②). The most extensively researched polymeric material for nucleic acid delivery is the polyethyleneimine (PEI) polymer family. They are made up of branching or linear polycations that can combine with miRNA or siRNA to form nanoscale complexes that protect RNA and distribute it to cells. JetPEITM, a linear PEI derivative that is sold commercially, is frequently utilized for transfection of DNA, siRNA, and mRNA. Additionally, in situ transformation of RNA vaccines from PEI functionalized graphene oxide hydrogel has been successfully employed for cancer immunotherapy [49]. By inhibiting growth factor pleiotrophin in glioblastoma, targeted CRM197-PEG-PEI-based complexes for siRNA delivery in vivo demonstrate therapeutic efficacy [50]. Nevertheless, PEI is not biodegradable and is comparatively cytotoxic [51]. A biopolymer called chitosan is present in the exoskeleton of crustaceans. Because of its biodegradability, biocompatibility, and ability to increase permeability, chitosan and its variations have been created for the delivery of DNA and siRNA. Nevertheless, their clinical use is limited by their poor transfection efficiency [52]. PEI that has been grafted with chitosan has been produced with reduced toxicity and improved transfection effectiveness [53]. The first targeted nanoparticulate siRNA delivery technology to enter clinical trials for cancer was cyclodextrin polymer (CDP)-based nanoparticles. In patients with solid tumors, the self-assembling, CDP-based nanoparticle (designated as CALAA-01) targets the ribonucleotide reductase M2 mRNA [54, 55].

APPLICATIONS AND THERAPEUTIC USES OF RNA BASED THERAPEUTICS

Cancer Therapy

Oncogenes that cause unchecked cell proliferation are suppressed by RNA-based treatments. These treatments aid in the reduction of cancer cell proliferation by focusing on certain mRNA involved in tumor growth. To improve the efficacy of treatment, they are also being studied in conjunction with immunotherapy and chemotherapy [56–58].

- *Genetic Disorders:* Mutations that result in aberrant protein synthesis are the cause of many hereditary disorders. Through the production of missing functional proteins or the silencing of defective genes, RNA-based therapies aid in the control or correction of these deficiencies. For hereditary illnesses, this method provides a focused and customized treatment alternative [59–61].
- *Viral Infections:* By focusing on viral RNA or host elements necessary for viral proliferation, RNA-based treatments effectively prevent viral replication. These treatments help prevent

infection and the advancement of disease by preventing the production of viral proteins. They are being investigated for several viral illnesses [62, 63].

- *Neurological Disorders:* The development of neurological disorders is significantly influenced by aberrant gene expression. RNA-based treatments aid in controlling proteins implicated in the degeneration and injury of neurons. Neurodegenerative and uncommon neurological illnesses may be treated with this method [64, 65].
- *Cardiovascular Diseases:* RNA-based treatments being investigated for the control of genes linked to inflammation, vascular function, and cholesterol metabolism. They support better cardiovascular health and disease management by modifying these pathways [66, 67].

OVERCOMING CHALLENGES IN THE DEVELOPMENT OF RNA THERAPEUTICS

Successful RNA therapy still depends on how well RNA is delivered into the cytoplasm by getting past extracellular and intracellular obstacles. First, RNA cannot passively diffuse across the lipid bilayers due to its big, hydrophilic, negatively charged characteristics. RNA medications must avoid serum nucleases and scavenge macrophages in the reticuloendothelial system in addition to the physical barrier. Furthermore, RNA medications need to use receptor-mediated endocytosis to traverse the cell membrane and the extracellular matrix. A crucial technical issue is getting out of the endosome and releasing RNAs into the cytoplasm in a non-toxic way [68]. Consequently, different chemical changes [69, 70] and delivery formulation engineering [71, 72] have been investigated to address issues with pharmacokinetics and pharmacodynamics.

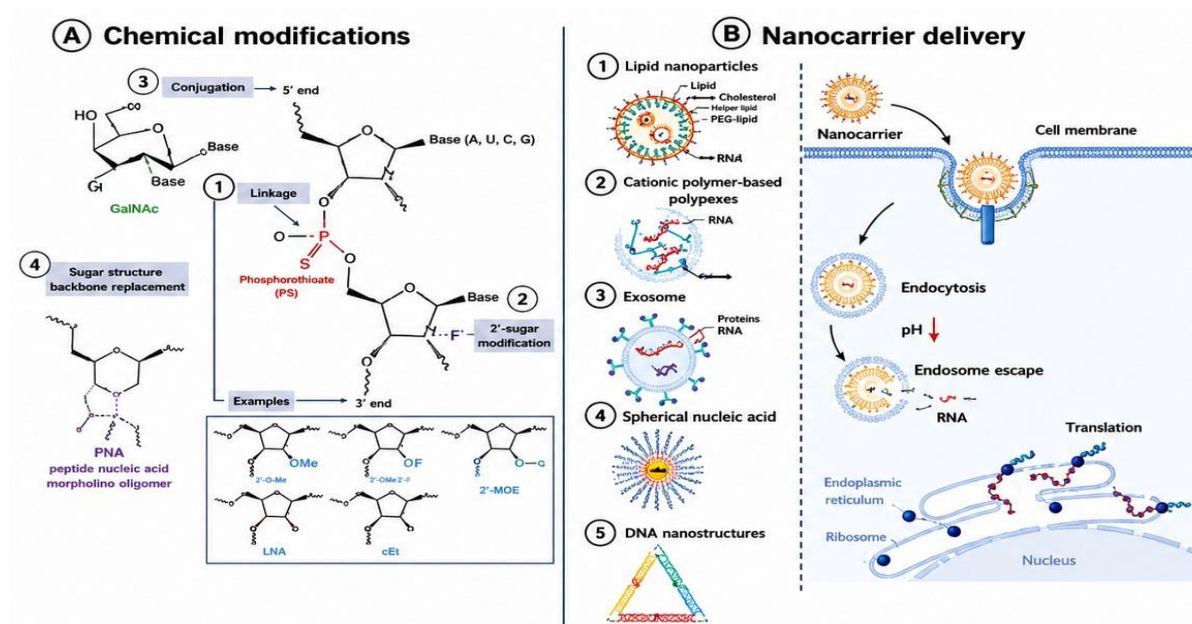


Figure 3. Overcoming challenges in the development of RNA therapeutics. A Common chemical modification. RNA-based drugs often have various chemical modifications, including 5'-and 3'-end conjugates, 2'-sugar substitution and inter-nucleoside linkage modifications. B Nanocarriers delivery strategies. Five representative nanocarriers are shown: (1) Lipid nanoparticles encapsulating nucleic acids. (2) Cationic polymers electrostatically bind to negatively charged nucleic acids to form polyplexes. (3) Engineered exosomes with aptamers or therapeutic RNAs on the outer surface. (4) Spherical nucleic acid nanoparticle consisting of an inorganic core coated in densely packed oligonucleotides attached by chemical linkages. (5) Self-assembled DNA cage tetrahedron nanostructure. Oligonucleotide drugs can be incorporated into the design of the DNA cage itself. Additional targeting ligands and polyethylene glycol (PEG) can be further conjugated to the nanostructure. These nanocarriers can deliver RNA molecules through binding to the cell membrane, endocytosis, endosome escape and RNAs are released in the cytoplasm and translation to proteins or incorporated into corresponding ribonucleoprotein complexes to silence target transcripts.

Viral Delivery

Adeno-associated virus (AAV), adenovirus (AdV), herpes simplex virus (HSV), retrovirus, and lentivirus are the most widely utilized viral vectors, which are among the most well researched RNA delivery systems. These vectors effectively transfer genes by introducing RNA into cells using viruses' inherent infection processes [73, 74]. Viral vectors have great potential for gene therapy and vaccine development since they can express RNA effectively and continuously. However, the therapeutic usefulness of viral vectors is limited by their immunogenicity and the hazards of genomic integration [75, 76]. For example, the usage of lentiviral and retroviral vectors has decreased because of the significant danger of insertional mutagenesis [77]. Although AdV and HSV transfer nucleic acids episomally, their usage is frequently linked to immunological reactions, severe inflammation, and difficulties with genetic modification. On the other hand, because of its low immunogenicity and ability to produce steady gene expression, especially in gene therapy and RNA delivery applications, AAV has become a preferred vector in research and clinical contexts [78]. In AD models, PKR depletion has demonstrated therapeutic promise [79–80].

Virus-Like Particle Delivery

Virus-like particles (VLPs) are non-infectious nanoscale entities made of viral proteins but without viral genetic material. Because of the hole in their structure, these VLPs can be formed in a range of systems, including mammals, plants, insects, and microbes, and they function as adaptable carriers for delivering bio- and nanomaterials, including medications, vaccinations, quantum dots, and imaging agents [81]. Furthermore, VLPs can effectively encapsulate and shield RNA, enabling highly precise targeted delivery with little chance of genome integration. To position VLPs as a safer substitute for conventional viral vectors in therapeutic applications, recent developments have concentrated on refining their design to increase delivery efficiency and cell-specific targeting [82, 83]. By modifying human and mouse PEG10 to package, secrete, and deliver particular RNAs, they created selective endogenous eNcapsidation for cellular delivery (SEND) (Figure 4). This technology effectively packaged mRNA containing the 5' and 3' untranslated regions (UTRs) of PEG10 flanking a gene of interest in recipient cells that expressed endogenous fusogen like SYNA. Target RNA transfer into recipient cells is facilitated by the pseudo-typed PEG10 VLPs, which are released within extracellular vesicles. SEND may be less immunogenic than currently existing viral vectors since it uses endogenous proteins [84].

LIPID NANOPARTICLE DELIVERY

Phospholipid bilayers make up liposomes, which are amphiphilic structures with a polar head group, a hydrophobic tail region, and a linker domain. They are essential in drug delivery systems because of their capacity to encapsulate and safeguard nucleic acids as well as their biocompatibility and adaptability [85, 86]. Additional surface modifications allow liposomes to accomplish targeted delivery, improve efficiency, and offer multifunctionality with low toxicity – all essential components for successful RNA delivery [87]. The liposome-like structures known as lipid nanoparticles (LNPs) are perfect for encasing and delivering different nucleic acids. Under physiological settings, the surface charge of clinical LNPs is usually close to neutral [88].

EXTRACELLULAR VESICLES DELIVERY

Because of their inherent capacity to encapsulate and shield RNA molecules, extracellular vesicles (EVs) have become a potential RNA delivery technology, allowing for targeted administration to particular cells [89]. They are highly appealing for therapeutic applications due to their biocompatibility and intrinsic targeting capabilities, particularly in effectively delivering RNA-based therapies [90]. By using this tactic, EVs can transfer amiR-4 to nearby cancer cells, impairing their antiviral defenses and increasing their susceptibility to viral assaults and small-molecule treatments. This strategy improves cancer treatment when used in conjunction with immunotherapies because it targets cancer cells more broadly while causing the least amount of damage to healthy tissues [91]. In a similar vein, Gu's group created leukocyte-derived EVs with retrovirus-like capsids to enhance systemic mRNA delivery to neurons. This method provides an immune-inert platform for gene therapy in neurological illnesses and

efficiently crosses the blood–brain barrier to deliver mRNA in a neuroinflammation mice model [92]. In a different work, Dong's group used microfluidic electroporation technique to create sEVs loaded with mRNA. They achieved improved mRNA delivery to cancer cells by conjugating targeting ligands, such as anti-CD71 and anti-PD-L1 antibodies, to the sEVs. This stimulated immune responses and provided a prospective treatment for drug-resistant and recurring cancers [93].

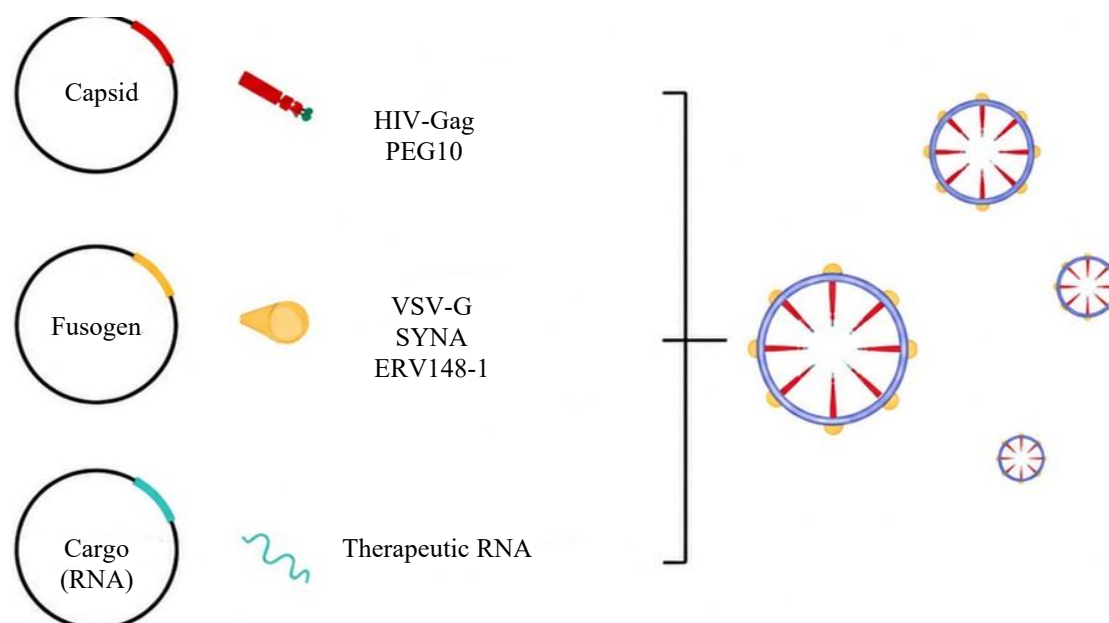


Figure 4. Schematic showing the components of SEND. SEND is a delivery platform that integrates an endogenous Gag homolog, a fusogen (glycoprotein), and cargo mRNA, allowing customization for specific applications.

OTHER NANOMATERIAL-BASED DELIVERY

Despite the successful clinical application of RNA therapies employing LNPs [94], there are still issues, especially regarding toxicity. Although LNPs' cationic lipids aid in RNA encapsulation, there is a potential of toxicity [95]. For example, harmful effects may result from interactions between cationic LNP components and enzymes such as protein kinase C [96, 97]. Cell lysis and necrosis can result from the disruption of cellular membranes caused by high amounts of these lipids [98, 99]. Numerous nanoparticle carriers, such as polymer-based (natural and synthetic), inorganic, hybrid nanoparticles, and biological methods utilizing peptide nano-assemblies, have been intensively researched to get beyond these restrictions [100].

- **Polymeric Nanoparticles:** Because they are highly modifiable and biocompatible, naturally occurring polymers – like hyaluronic acid and chitosan – are frequently used as mRNA carriers in drug delivery systems. Chitosan is unique among these due to its cationic charge and ease of modification, which promote surface adsorption and complex formation [101]. By adjusting variables including polymer length, degree of deacetylation, hyaluronic acid concentration, charge density, and nucleic acid composition, Soliman et al. showed that chitosan-based nanoparticles could achieve a transfection effectiveness of 60–65% in vitro [102]. Chitosan nanoparticles have been used to transport miRNA mimics, which are important biomarkers in cancer treatment, in addition to mRNA [103]. By modifying gene expression in vitro and in vivo, these miRNA/chitosan complexes target macrophages to treat cardiovascular disorders. Reverse cholesterol transfer was decreased in mice treated with miRNA/chitosan nanoparticles, indicating their potential to cure atherosclerotic lesions [104]. Additionally, adding heparin to chitosan nanoparticles improves the effectiveness of transfection. Under mildly acidic conditions (pH 4.5), polyplex swelling and network collapse caused heparin-loaded chitosan nanoparticles to release oligonucleotides in a pH-dependent manner, according to Pilipenko et al. In ARPE-19

cells, siRNA targeting vascular endothelial growth factor (VEGF) supplied using heparin-enriched chitosan nanoparticles demonstrated a 25% higher silencing efficacy than chitosan nanoparticles without heparin [105, 106].

- *Peptide-Derived Nanoparticles*: Cell-penetrating peptides (CPPs) are short, cationic peptides (5–30 residues) that can mediate the intracellular transport of a variety of biological cargos, including as genetic materials and big protein complexes, which normally have difficulty passing through cellular membranes. CPPs are interesting candidates for peptide-based RNA delivery platforms because they can cross membranes through energy-dependent or independent methods [107, 108]. To improve the effectiveness of mRNA transfection, Levaïc et al. created a library of peptide-like oligoaminoamides comprising synthetic amino acids, histidine modifications, and bio-reducible disulfide blocks [109].
- *Inorganic Nanoparticles*: Because of their perfect qualities – such as non-toxicity, hydrophilicity, biocompatibility, and high stability – inorganic materials are crucial for the distribution, packaging, and protection of RNA. Gold nanoparticles (AuNPs) are unique among them because of their bio-inertness, ease of production, and functionalization via electrostatic absorption or thiol-gold bonding. These nanoparticles, often referred to as spherical nucleic acids (SNAs), show improved stability, high cellular absorption efficiency, and extended therapeutic effectiveness when functionalized with thiolated siRNAs. Notably, SNAs can lower tumor burden and mute oncogenes without causing negative side effects by passing through the blood–brain and blood–tumor barriers [110]. This opens the door for clinical applications. Dissolvable nanoparticles, like calcium phosphate (CaP), disintegrate in acidic environments to release RNA cargo and lower the chance of tissue buildup. Systemic siRNA distribution to tumors is much improved by lipid-coated CaP nanoparticles [111]. Approved for imaging, iron oxide nanoparticles are multifunctional systems that combine imaging, siRNA delivery, and magnetically controlled targeting for gene therapy [112]. Because of their large surface area, porosity, and biodegradability, metal-organic frameworks (MOFs) provide effective RNA transport via coordination, encapsulation, or biomineralization. RNA is released following structural collapse, improving therapeutic efficacy [113].

FUTURE PERSPECTIVES

Recent developments in RNA delivery systems have demonstrated enormous potential for therapeutic uses, especially in the creation of vaccines and the management of illness. When it comes to enhancing the stability, specificity, and effectiveness of RNA-based treatments, platforms including viral vectors, LNPs, VLPs, and EVs each have unique benefits. Notably, LNPs have shown great efficacy in both the production of siRNA-based medications and the administration of mRNA vaccines, as evidenced by the success of COVID-19 vaccines. Each delivery method, however, has its own set of difficulties. For example, AAVs can only package up to 4.5 kb of DNA; new research shows that RNA molecules delivered by AAVs are usually shorter than 2000 nt, though it is still unknown how long RNA can fit into AAVs. Furthermore, the transduction effectiveness of AAV is dose-dependent; although greater doses can improve efficiency, they also raise immunogenicity and toxicity [114, 115]. By focusing on certain RNA sequences, these methods enable the up-regulation or down-regulation of particular mRNA expression and the inhibition of ncRNA functions. However, the biggest barrier to the general application of RNA-based strategies is the challenge of effectively delivering such medications to target organs and tissues other than the liver. Off-target binding is another [116].

- RNA-based treatments have advanced from laboratory studies to clinical trials.
- Numerous RNA medications and vaccines have already received approval for use in humans.
- Treatments for neurological conditions, viral infections, cancer, and genetic problems are being investigated in ongoing clinical studies.
- Stability, delivery, and off-target effects are current issues.
- It is anticipated that improvements in delivery methods, chemical alterations, and customized strategies would increase efficacy and safety.

With more accurate and focused therapies in the future, RNA therapeutics will probably play a significant part in contemporary medicine [117–120]. In conjunction with a transposable transgene given via rAAV, Zakas et al. investigated a unique in vivo delivery strategy based on an engineered transposase, Sleeping Beauty, supplied as an mRNA within an LNP [121]. As a result, bioengineered RNAi agents (BERAs), a recently created in vivo RNA agent with little to no post-transcriptional changes, have demonstrated promising application potential [122]. The creation of nanocarriers for the delivery of RNA drugs offers superior hepatic transport. More research is needed on non-liver systemically delivered nanomedicines. improvements in omics-based methods [123] and imaging techniques (such as electron microscopy, super-resolution fluorescence microscopy, single-particle tracking 3, etc.) [124]. For example, RNA transport in cardiac damage repair may be enhanced by the primate-specific endogenous retroviral envelope protein ERVH48-1, which sequesters SFRP2 to control human cardiomyocyte development [125].

CONCLUSION

RNA-based therapeutics have emerged as a transformative approach in modern medicine, offering precise regulation of gene expression and expanding the range of treatable diseases beyond the capabilities of conventional therapies. Significant advances in RNA technologies, including siRNA, miRNA, antisense oligonucleotides, mRNA therapeutics, and aptamers, have demonstrated promising applications in cancer, genetic disorders, infectious diseases, neurological conditions, and cardiovascular diseases. The successful development of mRNA vaccines and the approval of several RNA-based drugs have validated the clinical potential of these therapeutic strategies.

Despite these achievements, major challenges remain, particularly regarding RNA stability, immunogenicity, off-target effects, and efficient delivery to specific tissues. The development of advanced delivery systems – such as lipid nanoparticles, extracellular vesicles, viral vectors, virus-like particles, and novel nanomaterials – has significantly improved the safety and efficacy of RNA therapeutics. Continued innovation in chemical modifications and targeted delivery technologies is expected to further overcome existing limitations.

Overall, RNA-based drug delivery represents a rapidly evolving field with immense potential to revolutionize personalized and precision medicine. Future research focusing on enhancing delivery efficiency, minimizing adverse effects, and expanding therapeutic applications will be critical for translating emerging discoveries into widely accessible clinical treatments. As our understanding of RNA biology and nanotechnology continues to advance, RNA therapeutics are poised to play an increasingly important role in shaping the future of pharmaceutical care and disease management.

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